Autocrine Regulation of Keratinocytes: The Emerging Role of Heparin-Binding, Epidermal Growth Factor-Related Growth Factors

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Although originally conceived as a basis for malignant cell growth, autocrine signaling networks are currently known to be activated during tissue repair and with *in vitro* cultivation. In human epidermal keratinocytes, activation of the epidermal growth factor receptor by cognate ligands mediates the majority of the autonomous replicative capacity of these cells and is necessary to inhibit differentiation and apoptosis. The importance of heparin-binding growth factors in activation of this receptor was first suggested by the strong anti-proliferative effects of soluble heparin-like molecules on keratinocyte growth. This and related evidence led to the identification of amphiregulin as a major autocrine factor for keratinocytes. The binding of amphiregulin and its homolog, heparin-binding epidermal growth factor-like growth factor, to the receptor is potentially amplified by autoinduction and cross-signaling through epidermal growth factor-related polypeptides and by transmodulation of other ErbB-family receptors (HER-2, -3, -4) in cells expressing these receptors. Heparan sulfate proteoglycans and the tetraspanin family of membrane-associated proteins appear to act as cofactors in amphiregulin-driven mitogenesis mediated by the epidermal growth factor receptor, but amphiregulin's immunolocalization to keratinocyte nuclei and to filopodia may indicate other potentially novel effects. Following from the observation that amphiregulin is overexpressed in lesional psoriatic epidermis, the importance of amphiregulin in hyperproliferative skin diseases has been further supported by recent studies of the targeted expression of a transgene encoding keratin 14 promoter-driven human amphiregulin to the basal epidermis of mice. Founder transgenic mice displayed a morphologic and microscopic cutaneous phenotype that shares characteristics with psoriasis. Pharmacologic regulation of amphiregulin's expression and receptor signaling may eventually prove to be an effective strategy in the treatment of hyperproliferative skin diseases. ***Key words: amphiregulin/autocrine growth/epidermal growth factor receptor/heparin-binding epidermal growth factor-like growth factor. J Invest Dermatol 111:715–721, 1998***

The concept of autocrine cellular signaling was originally invoked to explain the unregulated, autonomous proliferation of malignant cells (Sporn and Todaro, 1980). It was postulated that dysregulated cellular proliferation results from endogenous production of growth-promoting polypeptides (transforming growth factors) and constitutive activation of cognate receptors, thereby supplanting the dependency on exogenous growth factors. The model further postulated that these mechanisms do not normally function in nontransformed cells except during embryogenesis, but that inappropriate and coordinate expression of these factors is activated with malignant transformation (Sporn and Todaro, 1980; Alexander and Currie, 1984). Notably, much of the investigations supporting these original tenets derived from *in vitro* studies on rodent fibroblasts. Revisions of the concept followed the discovery that autocrine pathways are expressed in normal cells and are inducible under various situations, such as during tissue repair (Stiles, 1984; Marikovsky *et al*, 1993; Liou *et al*, 1997) or when normal epithelial cells such as epidermal keratinocytes and mammary epithelial cells are cultured *in vitro* (Coffey *et al*, 1987a, b; Shipley *et al*, 1989; Cook *et al*, 1991a; Li and Shipley, 1991; Li *et al*, 1992; Piepkorn *et al*, 1994; Stoll *et al*, 1997). Following from our current understanding of autocrine regulation of various epithelial tissues and organs, autocrine dysregulation emerges as a potential mechanism contributing to the pathophysiology of those disease states that manifest aberrant hyperproliferation. Thus, the growth factors of autocrine regulatory networks hold promise as targets for therapeutic intervention through pharmacologic control of their expression, interaction with cognate cell surface receptors, or their coupled downstream signal transduction pathways.

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Abbreviations: AR, amphiregulin; EGFR, epidermal growth factor receptor; HB-EGF, heparin-binding epidermal growth factor.

THE EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING AXIS

Contrary to early claims that only malignantly transformed cells display autocrine-driven proliferation in culture (Sporn and Todaro, 1980; Alexander and Currie, 1984), we and others have shown that normal

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human keratinocytes exhibit autonomous (autocrine growth factor driven) proliferation when cultured at cell densities above 1×10^3 cells per cm^2 (Shipley *et al*, 1989; Cook *et al*, 1991a; Pittelkow *et al*, 1993; Piepkorn *et al*, 1994). With the exception of acidic fibroblast growth factor and keratinocyte growth factor, human keratinocytes appear to require binding of epidermal growth factor (EGF) or other family member ligands to the EGF receptor (EGFR, human EGFR-1, or HER-1) to sustain proliferation in culture (Rheinwald and Green, 1977; Finch *et al*, 1989; Shipley *et al*, 1989; Pittelkow *et al*, 1993). Current understanding of autocrine signaling in epidermal keratinocytes thus assigns a principal function to the EGFR/HER-1 and its specific ligands. The primary role of the EGF receptor-ligand system as an endogenous signaling axis in keratinocytes is reflected by the observation that blocking antibody against the EGFR inhibits greater than –90% of autocrine growth of these cells *in vitro* (Cook *et al* 1991b; Pittelkow *et al*, 1993; Piepkorn *et al*, 1994; Ristow, 1996; Peus *et al*, 1997). More recent investigation has also demonstrated that interruption of the activity of EGF-related autocrine growth factors in human keratinocytes by neutralizing antibody to the EGFR, or a specific inhibitor of the EGFR tyrosine kinase (PD 153035), not only causes cessation of keratinocyte proliferation, but also induces irreversible growth arrest and terminal differentiation (Peus *et al*, 1997). The correlation between keratinocyte proliferation and expression of the EGFR is further illustrated by immunolabeling, which localizes the receptor to the proliferative basal compartment in adult epidermis, as well as to all epidermal layers and appendages of fetal skin (Nanney *et al*, 1990; Sakai *et al*, 1994). EGFR null mice, moreover, exhibit severe epidermal atrophy, markedly diminished basal rates of epidermal keratinocyte replication, and premature differentiation (Miettinen *et al*, 1995; Sibilia and Wagner, 1995; Threadgill *et al*, 1995).

EGF and its homolog, transforming growth factor-α (TGF-α), were originally considered the principal ligands for the EGFR in keratinocytes (Rheinwald and Green, 1977; Wille *et al*, 1984; Coffey *et al*, 1987a, b; Pittelkow *et al*, 1988, 1989). The importance of heparin-binding autocrine factors in keratinocytes, however, first emerged from the observation that exogenous heparin-like glycosaminoglycans strongly inhibit the autonomous growth of these cells (Shipley *et al*, 1989; Cook *et al*, 1991a, 1992b; Piepkorn *et al*, 1994), whereas both EGF and TGF-α were shown to lack heparin-binding and heparin-mediated regulation in human keratinocytes (Cook *et al*, 1991b). Juxtacrine-mediated autonomous growth has also been implicated in normal keratinocytes as we have shown that colonies of at least 4–10 cells are required for the heparin-inhibitable, EGF-receptor-dependent clonal expansion of these cells in the absence of exogenous growth factors (Pittelkow *et al*, 1993). Subsequently, as the list of EGF homologs has expanded, several ligands have been identified with binding affinity for heparinoids. Specifically, an EGF-related ligand named amphiregulin (AR) has been implicated in mediating the autocrine growth and heparin-dependent growth inhibition of normal epithelial (e.g., epidermal or mammary) cells derived from ectoderm (Cook *et al*, 1991a; Li and Shipley, 1991, 1992b; Li *et al*, 1992; Piepkorn *et al*, 1994).

AR is a member of the EGF family with sequence homology within its carboxy-terminal domain to EGF and TGF-α (Shoyab *et al*, 1988, 1989; Plowman *et al*, 1990; Cook *et al*, 1991b) and is presently identified as a major autocrine factor responsible for the autonomous proliferation of human keratinocytes when cultured under serum free conditions in the absence of exogenous EGF or bovine pituitary extract (Cook *et al*, 1991b; Piepkorn *et al*, 1994). The homolog, heparin-binding EGF-like growth factor (HB-EGF) (Higashiyama *et al*, 1992), may also exert some autocrine effects in these cells (Hashimoto *et al*, 1994). By blocking antibody studies, AR and HB-EGF account for –70% (Piepkorn *et al*, 1994) and less than 30% (Hashimoto *et al*, 1994), respectively, of autocrine-dependent proliferation. The molar mitogenic potency of human recombinant AR with murine and human keratinocytes is also comparable with that of EGF (Shoyab *et al*, 1989; Cook *et al*, 1991b; Piepkorn *et al*, 1994).

Because HB-EGF shares with AR a strong sequence homology (Higashiyama *et al*, 1992), a common pathway of signal transduction through the EGFR (Higashiyama *et al*, 1992), and an apparent requirement for cell surface heparin-like glycosaminoglycans as cofactors in at least some cell lineages (Higashiyama *et al*, 1993; Aviezer and Yayon, 1994; Johnson and Wong, 1994; Piepkorn *et al*, 1994; Thompson *et al*, 1994; Cook *et al*, 1995b), it is presumed for the purposes of this overview that the functional activities of the two factors are similar and, perhaps, partly redundant. One curious difference, however, is that the mitogenic activity of HB-EGF in various cells including keratinocytes is variably potentiated by soluble heparinoids (Marikovsky *et al*, 1993; Cook *et al*, 1995a), in stark contrast to their attenuative effects on AR signaling (Cook *et al*, 1991b, 1992b, 1995a; Piepkorn *et al*, 1994); the enhancing effect of the HB-EGF depends on a conserved leucine residue (leu_{76}) near the carboxyl terminus, which is absent in the AR coding sequence (Cook *et al*, 1995a).

MOLECULAR BIOLOGY OF AMPHIREGULIN

At the genomic level, the single copy gene for AR maps to chromosome 4q13–21 and is partitioned into six exons covering 10.2 kb of genomic DNA (Plowman *et al*, 1990). As deduced from the cDNA clone, the translation product is a prepropeptide of 252 amino acids, predicting a mass without glycosylation of 25.9 kDa. The domain structure of the deduced precursor conforms to a type I transmembrane protein, with extracellular, membrane spanning, and cytoplasmic tail domains. The mature, secreted growth factor, originally considered to be either 78 or 84 residues in length, is embedded within the precursor and is processed from the ectodomain after membrane insertion (Plowman *et al*, 1990); however, recombinant AR isoforms with carboxy-terminal extensions have substantially higher mitogenic activities than the recombinant 84 residue molecule, suggesting that the actual terminus of native AR may be more distal than originally thought (Adam *et al*, 1995; Thompson *et al*, 1996). The soluble species contains an N-terminal, hydrophilic segment with two putative binding sites for heparin-like glycosaminoglycans, at positions 125–129 (KRKKK) and 140–143 (RKKK), and a 41-residue carboxy terminus with the EGF-like motif of six conserved cysteinyl residues that are presumed to form three disulfide loops (Plowman *et al*, 1990). Cellular processing and secretion of AR are modulated via extensive post-translational modification by N-linked glycosylation, creating isoforms with varying biologic properties (Johnson *et al*, 1993b; Thome and Plowman, 1994; Martinez-Lacaci *et al*, 1996). The release of multiple soluble AR isoforms of varying molecular mass has been shown to occur from a common, membrane-anchored precursor at the plasma membrane by a phorbol ester-regulated process that is sensitive to a metalloprotease inhibitor (Brown *et al*, 1998) (**Fig 1**).

Soluble, secreted AR apparently exerts its tyrosine phosphorylation and mitogenic effects largely, if not exclusively, through binding and activation of the 170 kDa EGFR (Johnson *et al*, 1993a; Piepkorn *et al*, 1994; Peus *et al*, 1997; Pittelkow *et al*, unpublished observations) (**Fig 1**). In support of this mechanism of action, AR and the EGFR partially colocalize by dual immunolabeling in cultured keratinocytes to sites of intercellular contacts (Nylander *et al*, 1998). Other effects of AR, however, may be mediated by separate pathways because the factor segregates under some growth conditions to the nucleus by scanning laser confocal microscopy of the immunolabeled cells, without evidence for coordinate intranuclear targeting of the EGFR (Nylander *et al*, 1998). Moreover, immunolabeled AR decorates filopodia at the outer leading edges of keratinocyte colonies, suggesting matrix interactions (Nylander *et al*, 1998).

Signal transduction following ligand activation of the EGFR tyrosine kinase involves broadly conserved downstream effectors (van der Geer *et al*, 1994). Ligand engagement induces receptor dimerization, activates intrinsic tyrosine kinase, and stimulates trans- and autophosphorylation at specific tyrosyl residues of the receptor. Phosphotyrosines are bound by the adapter molecule GRB2 via its src homology 2 (SH2) domain; coupling of the signal through phosphorylation of a factor, SOS, stimulates guanine nucleotide exchange activity, activating the ras proto-oncogene product. Downstream effects on transcriptional factors that regulate gene expression are controlled, in part, by the mitogen-activated protein or extracellular regulated kinase cascade that is linked to ras activation. There is complex redundancy and cross-regulation between the factors that act through the EGFR (Barnard *et al*, 1994;

Ebert *et al*, 1994; Hashimoto *et al*, 1994; Sehgal *et al*, 1994). Signal amplification is achieved by autoinduction of AR mRNA and protein, as well as by cross-signaling from EGF, TGF-α, or HB-EGF and potentially by transmodulation from the ErbB-(HER)-2, -3, and -4 receptors, which are homologs of the EGFR (Barnard *et al*, 1994; Beerli and Hynes, 1996) (**Fig 2**). Whether downstream signal transduction pathways via EGFR activation are qualitatively different in relation to which EGF-related ligands engage the receptor is not clearly defined at present.

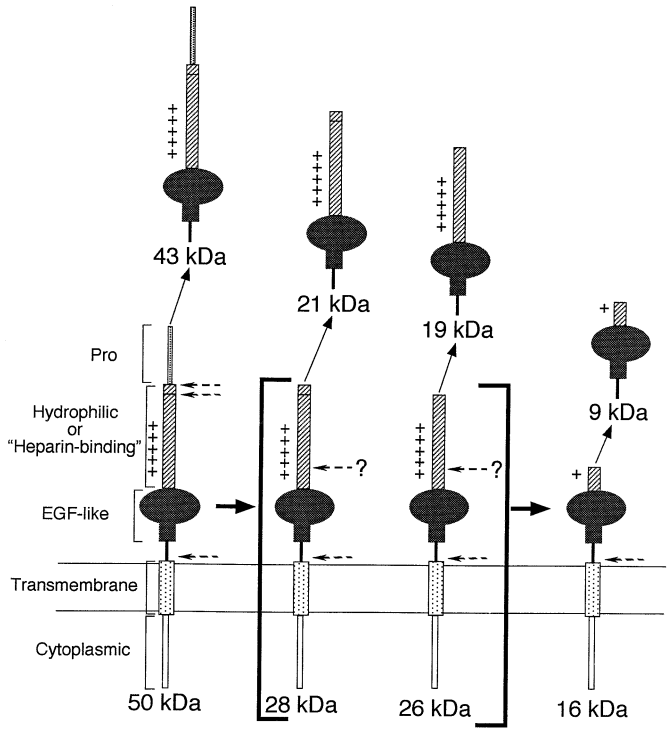

Figure 1. Membrane-anchored pro-AR is proteolytically processed at the plasma membrane into multiple soluble isoforms. The transmembrane precursor is partitioned into five structural domains. The common processing site within the carboxyl-terminus of the EGF-like domain is indicated by the dashed arrows at the plasma membrane. Cleavage of the pro-domain and at additional candidate sites (*arrows*) within the hydrophilic domain creates secretory isoforms that vary in relative mass from 9 to 43 kDa.

AMPHIREGULIN SIGNALING COMPLEX

Activation of the EGFR by TGF-α and EGF is independent of any requirement for cellular proteoglycans as obligate cofactors; in contrast, experimental observations indicate that receptor activation by AR (Johnson and Wong, 1994; Piepkorn *et al*, 1994; Cook *et al*, 1995b) and, at least in some cell types, HB-EGF (Higashiyama *et al*, 1993), requires endogenous membrane heparan sulfate proteoglycans as obligate cofactors. In other cell types such as mouse fibroblasts and keratinocytes, however, the signaling of HB-EGF might occur through alternative proteoglycan independent pathways utilizing receptors other than those that interact with either EGF or TGF-α (Cook *et al*, 1995b) (**Fig 2**). In the case of AR effects on cultured human keratinocytes, a trimolecular signaling complex of ligand, proteoglycan, and the EGFR has been hypothesized (Johnson and Wong, 1994; Piepkorn *et al*, 1994; Cook *et al*, 1995b) (**Fig 2**). As such, the molecular interactions may be analogous to fibroblast growth factors (FGF) signaling through their specific tyrosine kinase receptors. Extrapolating from the model of FGF signal transduction, the binding of AR to the heparan sulfate chains of a membrane proteoglycan could be mediated by the two strongly basic loci within the amino terminus of the mature factor (Plowman *et al*, 1990), which would serve to present the ligand to the EGFR. The basic residues, however, may also function as recognition sequences for nuclear targeting (Plowman *et al*, 1990), as reflected in reports of the nuclear localization of AR in several cell types, including human keratinocytes (Johnson *et al*, 1991, 1992; Modrell *et al*, 1992; Normanno *et al*, 1993; Piepkorn *et al*, 1995). The evidence at present is consistent with at least two alternative models for AR and/or HB-EGF signaling at the cell surface. As with FGF signaling (Olwin and Rapraeger, 1992; Kan *et al*, 1993), membrane heparan sulfate proteoglycans could bind growth factor and present it to the receptor in an optimal conformation for activating the receptor. Alternatively, heparan sulfate proteoglycan with its multivalent glycosaminoglycan chains may bridge contiguous receptors directly or via the growth factor as linker molecule, promoting receptor oligomerization. Potential regulatory targets under these alternate models include variations in affinity between the glycosaminoglycan and growth factor, due in part to changes in heparan sulfate structure related to the growth status of the cells, or enzymatic deglycosylation of heparan sulfate proteoglycans occurring on the cell surface, which could strongly downregulate signal transduction (Piepkorn *et al*, 1987, 1988, 1990, 1994).

It is not clear which specific proteoglycans regulate signaling of AR and other heparin-binding growth factors. Syndecans, a class of hybrid membrane proteoglycans, can bind FGF *in vitro* (Chemousov and Carey, 1993; Aviezer *et al*, 1994) and can support the signaling of

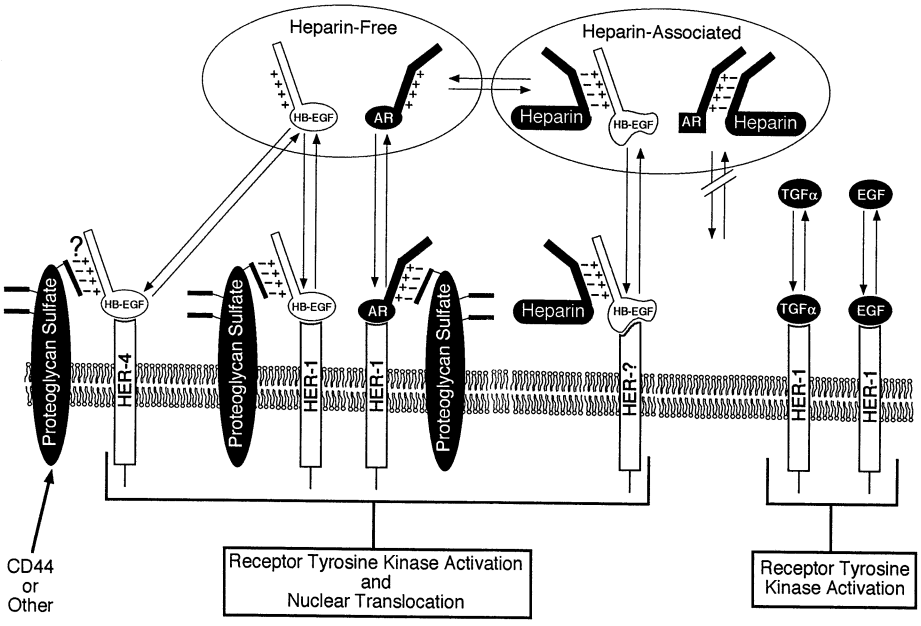

Figure 2. The signaling of heparin-binding, EGF-like ligands through the HER-family of receptors is regulated by heparinoids. Activation of the intrinsic tyrosine kinase domains of EGFR (HER-1), HER-4, and perhaps other homologs initiates signal transduction following ligation of EGF, TGF-α, AR, or HB-EGF. In contrast to the signaling of EGF and TGF-α (far right), the interaction of AR and HB-EGF with cognate receptors requires a membrane heparan sulfate proteoglycan as an obligate cofactor, depicted as a hypothetical ternary signaling complex. Soluble heparin-like molecules act as competitive inhibitors of the proteoglycan for binding of growth factor, thereby abrogating signaling through HER-1 and, in the case of HB-EGF, HER-4. There is, however, evidence that HB-EGF may also ligate an uncharacterized receptor and signal without an obligate proteoglycan cofactor.

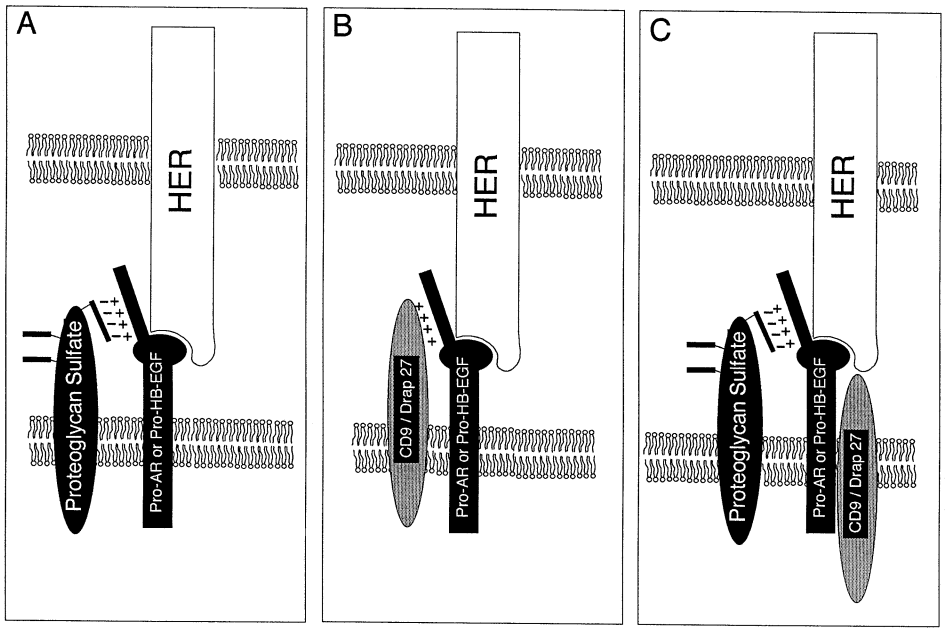

Figure 3. Juxtracrine receptor activation of keratinocytes is mediated via pro-AR or pro-HB-EGF, in association with heparan sulfate proteoglycans and/or CD9/Drap 27. Signaling between adjacent keratinocytes involves the ligation of membrane-intercalated growth factors, depicted as complexes with either heparan sulfate proteoglycan (*A*), CD9/Drap 27 (*B*), or both (*C*), to receptors on adjacent keratinocytes.

FGF-2 through its cognate receptor (Steinfeld *et al*, 1996). Among the various heparan sulfate proteoglycans synthesized by keratinocytes, alternatively spliced CD44 isoforms are plausible candidates for obligate cofactors in the membrane-based signaling of AR and other heparin-binding growth factors. Keratinocytes synthesize high molecular weight isoforms spliced with variant exon V3 (Kugelman *et al*, 1992), the splicing of which in recombinant systems is associated with glycanation by heparan sulfate chains and with the acquisition of affinity for heparin-binding growth factors in ligand blot assays (Bennett *et al*, 1995). The candidate coreceptor role of CD44 isoforms in AR signaling has been evaluated by correlating its topographical distribution with those of AR and the EGFR. By dual immunostaining and imaging with scanning laser confocal microscopy, CD44 and the EGFR colocalize with AR to sites of intercellular contacts in the interior of keratinocyte colonies (Nylander *et al*, 1998). This coordinate segregation is consistent with a concerted function. At these sites, signaling of the soluble growth factor or its membrane-anchored precursor is presumably mediated via ligation of the EGFR, with the CD44 proteoglycan, or another proteoglycan, serving as an obligate cofactor.

Another cell surface-associated family of proteins, transmembrane 4 (TM4) or tetraspanin super family that include CD9, has also been shown to be involved in the upregulation of membrane-anchored HB-EGF and AR (Inui *et al*, 1997; Maecker *et al*, 1997). Juxtacrine growth activity is enhanced by coexpression of CD9 with pro-HB-EGF or pro-AR in mouse L-cells, and anti-CD9 antibodies neutralize juxtacrine activity of pro-HB-EGF (**Fig 3**). Approximately half the growth activity of cultured keratinocytes is inhibited by anti-CD9 antibody, and CD9 coprecipitates with pro-HB-EGF and pro-AR (Inui *et al*, 1997).

ALTERNATE SITES OF CELLULAR FUNCTION

It has been postulated that basic FGF and possibly other heparin-binding growth factors, are ligated to their receptors on the cell surface and routed by endocytosis to cytoplastmic and intranuclear sites as molecular complexes with heparan sulfate glycosaminoglycans (Amalric *et al*, 1994; Hawker and Granger, 1994). In studies evaluating the possibility that AR is similarly routed, the nuclear localization of the growth factor was observed to be accentuated in growth-arrested keratinocytes (Nylander *et al*, 1998). This correlates with the prior observation that it is predominantly restricted to the occasional nuclei of spinous keratinocytes in normal adult human epidermis (Piepkorn *et al*, 1995), but its expression is markedly upregulated in the cytoplasm and cell membranes in hyperproliferative lesions (Piepkorn, 1996). Nuclear transport of the growth factor may therefore serve as an alternative signaling pathway exerting an attenuative effect on growth in some cell types. In contrast, the ability of rat AR to mediate nuclear targeting-dependent mtitogenic activity in the apparent absence of EGF-receptor tyrosine kinase activity (Kimura, 1993) argues that nuclear localization may be an important component of AR-dependent mitogenic signaling in other cell types.

Autocrine signaling pathways may mediate, in part, the effects of cell–matrix interactions on the cellular phenotype (Nakamura *et al*, 1995). The growth and motility of keratinocytes, as with other nontransfiormed cells, are strongly controlled by intercellular and cell–matrix contacts. Heparin-binding growth factors have recently been linked to adhesive mechanisms (Nakamura *et al*, 1995). Integrins, which are heterodimers of α and β subunits, mediate many processes related to cell adhesion and motility, matrix and cytoskeletal reorganization, and signal-transducing phosphorylation events through tightly coordinated binding of their extracellular domains to insoluble proteins of the extracellular matrix or to counter receptors on adjacent cells (Schwartz *et al*, 1995). Additional effects are mediated via second messengers (e.g., PKC, PTK, mitogen-activated protein kinases) that activate early response genes. Of the β1 integrins, the $\alpha 3\beta 1$ heterodimer specifically mediates intercellular adhesion during the transition from migratory to stationary phenotype in cultured keratinocytes**,** which is associated with unique signaling events and growth inhibition (Symington *et al*, 1993). Moreover, HB-EGF, CD9 (the diphtheria toxin receptor-associated protein, DRAP27), and $\alpha 3\beta 1$ integrin coimmunoprecipitate and cross-link *in situ* and colocalize by immunolabeling at cell contact sites, coordinately with α-catenin, vinculin, and other cytoskeletal components (Nakamura *et al*, 1995). Thus, the evidence now establishes a direct link between HB-EGF and cell adhesion molecules. This link provides a potential means to coordinately regulate autocrine signaling by AR and HB-EGF through the EGFR during matrix and intercellular reorganization.

AUTOCRINE NETWORKS IN SKIN DISORDERS

Preliminary characterizations have been made of AR expression at the mRNA and protein levels in developing skin and cutaneous disorders. The expression of AR protein is developmentally regulated in a spatial- and time-dependent fashion during human skin morphogenesis (Piepkorn *et al*, 1995). Immunolabeling and northern analyses have indicated that AR is low to undetectable in normal adult epidermis and markedly over-expressed in some neoplastic and non-neoplastic hyperproliferative disorders of the epidermis (Cook *et al*, 1992a; Hardas *et al*, 1992; Elder *et al*, 1993; Piepkorn, 1996). These include appendageal tumors, actinic keratoses, verrucae, and squamous cell carcinomas (Piepkorn, 1996). There is especially strong immunostaining in the cytoplasm of keratinocytes within psoriatic epidermis, in contrast to the sparse, focal labeling restricted to keratinocyte nuclei in normal

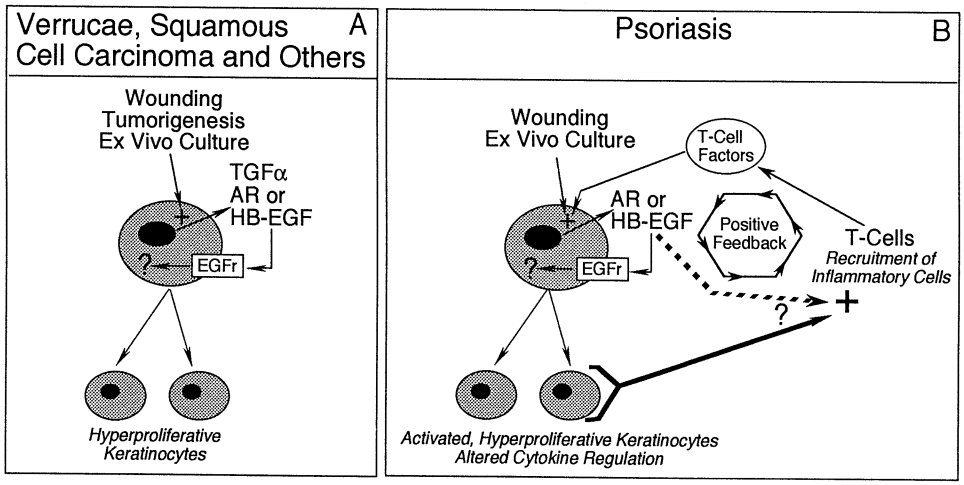

Figure 4. Induction of keratinocyte autocrine growth factors under pathologic stimuli mediates the hyperproliferative phenotype. AR, HB-EGF, and TGF-α are upregulated by uncharacterized mechanisms during epidermal wounding, with neoplastic development, and during *ex vivo* culturing, resulting in pathologic hyperproliferation of epidermal keratinocytes (*A*). In the case of psoriasis, there is evidence that autocrine induction by wounding (the Koebner reaction) is associated with the parallel recruitment of an inflammatory response (*B*). T cell derived factors may then further activate autocrine signaling in a positive feedback loop.

skin, which correlates well with the dramatically elevated mRNA expression of AR in involved psoriatic skin (Cook *et al*, 1992a; Piepkorn, 1996). This observation raises the possibility that upregulation of the AR signaling axis contributes to the phenotypic expression of psoriasis.

A mediating function for aberrant expression of AR in the psoriatic phenotype has been further tested by the recent construction and expression of a transgene encoding a keratin 14 promoter-driven human AR gene in the basal epidermis of mice (Cook *et al*, 1997). Affected mice demonstrated limited life-spans, prominent scaling and erythematous skin with alopecia, and occasional papillomaltous epidermal growths. Histologic examination revealed extensive areas of marked hyperkeratosis with focal parakeratosis, acanthosis, dermal and epidermal lymphocytic and neutrophilic infiltration, and dilated blood vessels within the papillary dermis. Our recent results have revealed that AR may exert activities in the skin that are distinct from that of transgenic TGF-α or other cytokines (IL-1α, TNF-α, IFN-χ, IL-6), and induces a cutaneous pathologic state with many similarities to psoriasis. Our observations also establish a link between the keratinocyte EGF receptor-ligand axis and psoriatic inflammation. Collectively, these results suggest that aberrant expression of AR in the epidermis could represent a critical step in the development of psoriatic lesions. Thus, the K14-AR transgenic mouse emerges as a potential model system for study of the psoriatic phenotype.

AR and HB-EGF may also mediate critical events in the cutaneous wounding response, including re-epithelialization and epidermal barrier function (Liou *et al*, 1997; Stoll *et al*, 1997). AR and HB-EGF transcripts are rapidly and strongly induced in skin organ culture, and these effects were markedly inhibited by the addition of neutralizing antibody to the EGFR or a potent EGFR-specific tyrosine kinase inhibitor (PD 153035) (Stoll *et al*, 1997). *In vivo* studies involving the disruption of the epidermal barrier by tape-stripping or acetone treatment showed a marked (12–30-fold) increase of AR, but not TGF-α, mRNA in murine epidermis (Liou *et al*, 1997). Collectively, these findings may provide an explanation for the role of autocrine growth factors in epidermal repair as well as the dysregulated expression and activity that contribute to the exaggerated isomorphic (Koebner) response in psoriasis (Cook *et al*, 1997) (**Fig 4**).

CONCLUSIONS

The signaling axis of the EGFR and its specific ligands plays a pivotal role in the autonomous proliferative capacity of cultured keratinocytes. Alternatively spliced CD44 proteoglycans as well as other membrane-associated proteins (CD9) and proteoglycans are candidate cofactors in this signaling pathway. Whereas AR and HB-EGF are principal ligands mediating autocrine growth, distinct functions for AR are suggested by its alternate sites of segregation to keratinocyte nuclei and to filopodia. The nuclear distribution implies transcriptional effects, which could correlate with both growth inhibition in human keratinocytes and mitogenic stimulation in other cell types. These speculative mechanisms may correspond to the original prediction from growth assays with transformed cells that AR mediates bifunctional effects (Shoyab *et al*, 1988) or other biologic properties distinct from that of either TGF-α or EGF, such as cell density-dependent mitogenic responsiveness and regulation by heparin or heparin-like factors (Cook *et al*, 1991a, b). Additional functions for the growth factor, probably in the form of membrane-anchored pro-AR, as a transducer of matrix interactions are suggested by the immunolabeling of filopodia at the outer leading edge of keratinocyte colonies (Piepkorn *et al*, 1995; Nylander *et al*, 1998). The validity of this suggestion is supported by the observation that HB-EGF coimmunoprecipitates and cross-links with α3β1 integrin (Nakamura *et al*, 1995). What the actual growth regulatory consequences might be of this physical interaction of an autocrine growth factor with an integrin-class matrix receptor remain to be elucidated. Moreover, we have recently reported that most of the clinical and histologic features of psoriasis can be produced by transgenically expressing AR in the basal epidermis of mice. These results link the aberrant activity of the epidermal keratinocyte EGF receptor-ligand system to the cutaneous inflammatory component of psoriasis. Other investigations have established that AR, HB-EGF, and TGF-α activity may play important roles in cutaneous wound healing, epidermal development, and epidermal tumor development. Thus, further investigations are warranted to determine the significance of these growth factors in both normal and abnormal skin physiology.

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REFERENCES

Adam R, Drummond DR, Solic N, Holt SJ, Sharma RP, Chamberlin SG, Davies DE: Modulation of the receptor binding affinity of amphiregulin by modification of its carboxyl terminal tail. *Biochim Biophys Acta* 1266:83–90, 1995

Alexander P, Currie G: Concomitant synthesis of growth factors and their receptors – an aspect of malignant transformation. *Biochem Pharmacol* 33:941–943, 1984

Amalric F, Bouche G, Bonnet H, Brethenou P, Roman AM, Truchet I, Quarto N: Fibroblast growth factor-2 (FGF-2) in the nucleus: Translocation process and targets. *Biochem Pharmacol* 47:111–115, 1994

Aviezer D, Yayon A: Heparin-dependent binding and autophosphorylation of epidermal growth factor (EGF) receptor by heparin-binding EGF-like growth factor but not by EG17. *Proc Natl Acad Sci USA* 91:12173–12177, 1994

Aviezer D, Levy E, Safran M, *et al*: Differential structural requirements of heparin and heparan sulfate proteoglycans that promote binding of basic fibroblast growth factor to its receptor. *J Biol Chem* 269:114–121, 1994

Barnard JA, Graves-Deal R, Pittelkow MR, *et al*: Auto- and cross-induction within the mammalian epidermal growth factor-related peptide family. *J Biol Chem* 36:22817–22822, 1994

Beerli RR, Hynes NE: Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities. *J Biol Chem* 271:6071–6076, 1996

Bennett KL, Jackson DG, Simon X, *et al*: CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. *J Cell Biol* 128:687–698, 1995

Brown CL, Meise KS, Plowman GD, Coffey RJ, Dempsey PJ: Cell surface ectodomain cleavage of human amphiregulin precursor is sensitive to a metalloprotease inhibitor. *J Biol Chem* 273:17258–17268, 1998

Chemousov MA, Carey DJ: N-Syndecan (syndecan 3) from neonatal rat brain binds basic fibroblast growthfiactor. *J Biol Chem* 268:16810–16814, 1993

Coffey W Jr, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses BL, Pittelkow MR: Production and autoinduction of transforming growth factor-α in human keratinocytes. *Nature* 328:817–820, 1987a

Coffey RJ, Goustin AS, Soderquist AM, Shipley GD, Wolfshol J, Carpenter G, Moses HL: Transforming growth factor α and β expression in human colon cancer lines: Implications for an autocrine model. *Cancer Res* 47:4590–4594, 1987b

Cook PW, Pittelkow MR, Shipley GD: Growth factor-independent proliferation of normal human neonatal keratinocytes: production of autocrine- and paracrine-acting mitogenic factors. *J Cell Physiol* 146:277–289, 1991a

Cook PW, Mattox PA, Keeble WW, *et al*: A heparin sulfate-regulated human keratinocyte autocrine factor is similar or identical to amphiregulin. *Mol Cell Biol* 11:2547–2557, 1991b

Cook PW, Pittelkow MR, Keeble WW, Graves-Deal R, Coffey RJ, Shipley GD: Amphiregulin messenger RNA is elevated in psoriatic epidermis and gastrointestinal carcinomas. *Cancer Res* 52:3224–3227, 1992a

Cook PW, Mattox PA, Keeble WW, Shipley GD: Inhibition of autonomous human keratinocyte proliferation and amphiregulin mitogenic activity by sulfated polysaccharides. *In Vitro Cell Dev Biol* 28A:218–222, 1992b

Cook PW, Damm D, Garrick BL, *et al*: Carboxyl-terminal truncation of $Leucine_{76}$ converts heparin-binding EGF-like growth factor from a heparin-enhancible to a heparin-suppressible growth factor. *J Cell Phyiol* 163:407–417, 1995a

Cook PW, Ashton NW, Karkaria CE, Siess DC, Shipley GD: Differential effects of a heparin antagonist (hexadimethrine) or chlorate on amphiregulin, basic fibroblast growth factor, and heparin-binding EGF-like growth factor activity. *J Cell Physiol* 163:418–429, 1995b

Cook PW, Piepkorn M, Clegg CH, Plowman GD, DeMay JM, Brown JR, Pittelkow MR: Transgenic expression of the human amphiregulin gene induces a psoriasis-like phenotype. *J Clin Invest* 100:2286–2294, 1997

Ebert M, Yokoyama M, Kobrin MS, *et al*: Induction and expression of amphiregulin in human pancreatic cancer. *Cancer Res* 54:3959–3962, 1994

Elder JT, Xia L-Q, Majmudar G, Johnson T: Selective overexpression of amphiregulin in cutaneous squamous and basal cell carcinoma. *J Invest Dermatol* 100:492, 1993

Finch PW, Rubin JS, Mike T, Ron D, Aaronson SA: Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science* 245:752–755, 1989

van der Geer P, Hunter T, Lindberg RA: Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol* 10:251–337, 1994

Hardas B, Yang Q, Elder M. Regulation of amphiregulin, a heparin-binding, EGF-like growth factor, in human skin. *J Invest Dermatol* 98:575A, 1992

Hashimoto Y, Higashiyama S, Asada H, *et al*: Heparin-binding epidermal growth factor-like growth factor is an autocrine growth factor for human keratinocytes. *J Biol Chem* 269:20060–20066, 1994

Hawker JR Jr, Granger FU: Nuclear accumulation of exogenous basic fibroblast growth factor in endothelial, fibroblast, and myoblast cell lines results in diverse biological responses. *In Vitro Cell Dev Biol* 30A:653–663, 1994

Higashiyama S, Abraham JA, Klagsbrun M: Heparin-binding EGF-like growth factor stimulation of smooth muscle cell migration: dependence on interactions with cell surface heparan sulfate. *J Cell Biol* 122:933–940, 1993

Higashiyama S, Lau K, Besner GE, Abraham JA, Klagsbrun M: Structure of heparin-binding EGF-like growth flactor. *J Biol Chem* 267:6205–6212, 1992

Inui S, Higashiyama S, Hashimoto K, Higashiyama M, Yoshikawa K, Taniguchi N: Possible role of coexpression of CD9 with membrane-anchored heparin-binding EGF-like growth factor and amphiregulin in cultured human keratinocyte growth. *J Cell Phys* 171:291–298, 1997

Johnson GR, Wong L: Heparan sulfate is essential to amphiregulin-induced mitogenic signaling by the epidermal growth factor receptor. *J Biol Chem* 269:27149–27154, 1994

Johnson GR, Saeki T, Auersperg N, Gordon AW, Shoyab M, Salomon DS, Stromberg K: Response to and expression of amphiregulin by ovarian carcinoma and normal ovarian surface epithelial cells: Nuclear localization of endogenous amphiregulin. *Biochem Biophys Res Commun* 180:481–488, 1991

Johnson GR, Saeki T, Gordon AW, Shoyab M, Salomon DS, Stromberg K: Autocrine action of amphiregulin in a colon carcinoma cell line and immunocytochemical localization of amphiregulin in human colon. *J Cell Biol* 118:741–751, 1992

Johnson GR, Kannan B, Shoyab M, Stromberg K: Amphiregulin induces tyrosine phosphorylation of the epidermal growth factor receptor and p185erbB2. Evidence that amphiregulin acts exclusively through the epidermal growth factor receptor at the surface of human epithelial cells. *J Biol Chem* 268:2924–2931, 1993a

Johnson GR, Prigent SA, Gullick WJ, Stromberg K: Characterization of high and low molecular weight forms of amphiregulin that differ in glycosylation and peptide core length. *J Biol Chem* 268:18835–18843, 1993b

Kan M, Wang F, Xu J, Crabb JW, Hou J, McKeehan WL: An essential heparin-binding domain in the fibroblast growth factor receptor kinase. *Science* 259:1918–1921, 1993

Kimura H: Schwannoma-derived growth factor must be transported into the nucleus to exert its mitogenic activity. *Proc Natl Acad Sci USA* 90:2165–2169, 1993

Kugelman LC, Ganguly S, Haggerty JG, Weissman SM, Milstone LM: The core protein of epican, a heparansulfate proteoglycan on keratinocytes, is an alternative form of CD44. *J Invest Dermatol* 99:381–385, 1992

Li S, Shipley GD: Expression of multiple species of basic fibroblast growth factor niRNA and protein in normal and tumor-derived mammary epithelial cells in culture. *Cell Growth Diff* 2:195–202, 1991

Li S, Plowman GD, Buckley SD, Shipley GD: Heparin inhibition of autonomous growth implicates amphiregulin as an autocrine growth factor for normal human mammary epithelial cells. *J Cell Physiol* 153:103–111, 1992

Liou A, Elias PM, Grunfeld C, Feingold KR, Wood LC: Amphiregulin and nerve growth factor expression are regulated by barrier status in murine epidermis. *J Invest Derm* 108:73–77, 1997

Maecker HT, Todd SC, Levy S: The tetraspanin superfamily: molecular facilitators. *FASEB J* 11:428–442, 1997

Marikovsky M, Breuing K, Liu PY, *et al*: Appearance ofheparin-binding EGF-like growth factor in would fluid as a response to injury. *Proc Natl Acad Sci USA* 90:3889–3893, 1993

Martinez-Lacaci I, Johnson GR, Salomon DS, Dickson RB: Characterization of a novel amphiregulin-related molecule in 12-O-tetradecanoylphorbol-13-acetate-treated breast cancer cells. *J Cell Physiol* 169:497–508, 1996

Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R: Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376:337–341, 1995

Modrell B, McDonald VL, Shoyab M: The interaction of amphiregulin with nuclei and putative nuclear localization sequence binding proteins. *Growth Factors* 7:305–314, 1992

Nakamura K, Iwamoto B, Mekada E: Membrane-anchored heparin-binding EGF-like growth factor (HB-EGF) and diphtheria toxin receptor-associated protein (DRAP27) /CD9 form a complex with integrin α3β1 at cell-cell contact sites. *J Cell Biol* 129:1691–1705, 1995

Nanney LB, Stoscheck CM, King LE Jr, Underwood RA, Holbrook KA: Immunolocalization of epidermal growth factor receptors in normal developing human skin. *J Invest Dermatol* 94:742–748, 1990

Normanno N, Qi C-F, Gullick WJ, *et al*: Expression of amphiregulin, cripto-1, and heregulin alpha in human breast cancer cells. *Int J Oncol* 2:903–911, 1993

Nylander N, Smith LT, Underwood RA, Piepkorn M: Topography of amphiregulin expression in cultured human keratinocytes: colocalization with the epidermal growth factor receptor and CD44. *In Vitro Cell Dev Biol* 34:183–189, 1998

Olwin BB, Rapraeger A: Repression of myogenic differentiation by aFGF, bFGF, and K-FGF is dependent on cellular heparan sulfate. *J Cell Biol* 118:631–639, 1992

Peus D, Hamacher L, Pittelkow M: EGF-receptor tyrosine kinase inhibition induces keratinocyte growth arrest and terminal differentiation. *J Invest Derm* 109:751–756, 1997

Piepkorn M: Overexpression of amphiregulin, a major autocrine growth factor for cultured human keratinocytes, in hyperproliferative skin diseases. *Am J Dermatopathol* 18:165–171, 1996

Piepkorn M, Fleckman P, Carney H, Linker A: Glycosaminoglycan synthesis by proliferating and differentiated human keratinocytes in culture. *J Invest Dermatol* 88:215–219, 1987

Piepkorn M, Fleckman P, Carney H, Linker A: Glycosaminoglycan synthesis by human keratinocytes: cell growth and medium calcium effects. *J Invest Dermatol* 91:492–498, 1988

Piepkorn M, Fleckman P, Carney H, Hovingh P, Linker A: The distinctive pattern of proteoglycan and glycosaminoglycan free chain synthesis by cultured human epidermal keratinocytes. *J Invest Dennatol* 94:107–113, 1990

Piepkorn M, Lo Q, Plowman G: Amphiregulin-dependent proliferation of cultured human keratinocytes: autocrine growth, the effects of exogenous recombinant cytokine, and apparent requirement for heparin-like glycosarninoglycans. *J Cell Physiol* 159:114–120, 1994

Piepkorn M, Underwood RA, Henneman Q, Smith LT: Expression of amphiregulin is regulated in cultured human keratinocytes and in developing fetal skin. *J Invest Dermatol* 105:802–809, 1995

Pittelkow MR, Coffey RJ, Moses BL: Keratinocytes 1 produce and are regulated by transforming growth factors. In: Milstone LM, Edelson RL (ed.). *Endocrine, Metabolic and Immunologic Functions of Keratinocytes*, Vol 548. New York: Ann NY Academy of Science, 1988, pp. 211–224

Pittelkow MR, Lindquist PB, Abraharn RT, Graves-Deal R, Derynck R, Coffey RJ: Induction of transforming growth factor-α expression in human keratinocytes by phorbol esters. *J Biol Chem* 264:5164–5171, 1989

Pittelkow MR, Cook PW, Shipley GD, Derynck R, Coffey W Jr: Autonomous growth of human keratinocytes requires epidermal growth factor receptor occupancy. *Cell Growth Differentiation* 4:513–521, 1993

Plowman GD, Green JM, McDonald VL, Neubauer MG, Disteche CM, Todaro GJ, Shoyab M: The amphiregulin gene encodes a novel epidermal growth factor-related protein with turnorinhibitory activity. *Mol Cell Biol* 10:1969–1981, 1990

Rheinwald J, Green H: Epidermal growth factor and the multiplication of human epidermal keratinocytes. *Nature* 265:421–424, 1977

Ristow H-J: Studies on stimulation of DNA synthesis with epidermal growth factor and insulinlike growth factor-1 in cultured human keratinocytes. *Growth Regulation* 6:96–109, 1996

Sakai Y, Nelson KG, Snedeker S, Bossert NL, Walker W, McLachlan J, Di Augustine RP: Expression of epidermal growth factor in suprabasal cells of stratified squamous epithelia: implications for a role in differentiation. *Cell Growth Diff* 5:527–535, 1994

Schwartz MA, Schaller MD, Ginsberg MH:. Integrins. Emerging paradigms of signal transduction. *Annu Rev Cell Dev Biol* 11:549–599, 1995

Sehgal I, Bailey J, Hitzemann K, Pittelkow MR, Maiffle NJ: Epidermal growth factor receptordependent stimulation of amphiregulin expression in androgen-stimulated human prostate cancer cells. *Mol Biol Cell* 5:339–347, 1994

Shipley GD, Keeble WW, Hendrickson JE, Coffey RJ, Pittelkow MR: Growth of normal human keratinocytes and fibroblasts in serum-free medium is stimulated by acidic and basic fibroblast growth factor. *J Cell Physiol* 138:511–518, 1989

Shoyab M, McDonald VL, Bradley JG, Todaro GJ: Amphiregulin: a biflanctional growthmodulating glycoprotein produced by the phorbol 12-myristate 13-acetate-treated human breast adenocarcinoma cell line MCF-7. *Proc Natl Acad Sci USA* 85:6528–6532, 1988

Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ: Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science* 243:1074–1076, 1989

Sibilia M, Wagner E: Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 269:234–238, 1995

Sporn MB, Todaro GJ: Autocrine secretion and malignant transformation of cells. *N Engl J Med* 303:878–880, 1980

Steinfeld R, Van den Bergeh H, David G: Stimulation of fibroblast growth factor receptor-1 occupancy and signaling by cell surface-associated syndecans and glypican. *J Cell Biol* 133:405–416, 1996

Stiles CD: Autocrine control of growth? *Nature* 311:604–605, 1984

Stoll S, Ciarner W, Elder J: Heparin-binding ligands mediate autocrine epidermal growth factor receptor activation in skin organ culture. *J Clin Invest* 100:1271–1281, 1997

Symington BE, Takada Y, Carter WG: Interaction of integrins, $\alpha 3\beta 1$ and $\alpha 2\beta 1$: potential role in intercellular adhesion. *J Cell Biol* 120:523–535, 1993

Thome BA, Plowman GD: The heparin-binding domain of amphiregulin necessitates the precursor pro-region for growth factor secretion. *Mol Cell Biol* 14:1635–1646, 1994

Thompson SA, Higashiyama S, Wood K, *et al*: Characterization of sequences within heparin-binding EGF-like growth factor that mediate interaction with heparin. *J Biol Chem* 269:2541–2549, 1994

Thompson SA, Harris A, Hoang D, Ferrer M, Johnson GR: COOH-terminal extended recombinant amphiregulin with bioactivity comparable with naturally derived growth factor. *J Biol Chem* 271:17927–17931, 1996

Threadgill DW, Dlugosz AA, Hansen LA, *et al*: Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 269:230–234, 1995

Wille M Jr, Pittelkow MP, Shipley GD, Scott RE: Integrated control of growth and differentiation of normal human prokeratinocytes cultured in scrum-free medium: clonal analysis, growth kinetics, and cell cycle studies. *J Cell Physiol* 121:31–44, 1984